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TECHNICAL MANUSCRIPT 395

# HISTOCHEMICAL DEMONSTRATION OF INTESTINAL GLYCOSIDASES IN THE NEONATAL RAT

John R. Esterly

APRIL 1967



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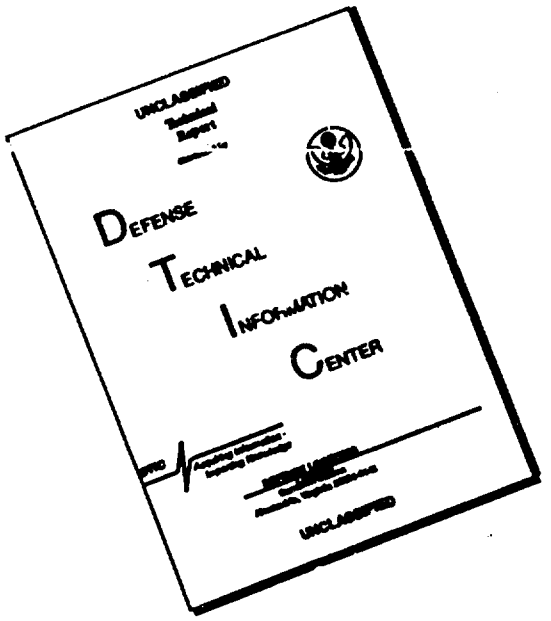


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# HISTOCHEMICAL DEMONSTRATION OF INTESTINAL GLYCOSIDASES IN THE NEONATAL RAT

John R. Esterly

Pathology Division
MEDICAL SCIENCES LABORATORY

Project 1L013001A91A

April 1967

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

## ACKNOWLEDGMENT

The advice and assistance of Dr. Bjarne Pearson are gratefully acknowledged.

## ABSTRACT

Indolyl substrates for β-D-galactosidase, β-D-glucuronidase, β-D-fucosidase, and β-D-glucosidase have been used to detect specific enzymatic activity in frozen sections of rat intestine. The animals ranged in age from newly born to 28 days. Specimens obtained from fetal, normal adult, and adult germ-free rats were used for controls. Reactions for all enzymes were demonstrated both in the epithelial cytoplasm and in reticular cells of the lamina propria. In the jejunum and ileum minimal reactivity for galactosidase (pH 5.4) in the mucosa was present at birth, but increased to high levels by the 4th day. After the 14th day it decreased again to adult levels. Prominent mononuclear cell staining was noted from late gestation through the neonatal period; it decreased somewhat thereafter. In contrast, activity in the colon was less intense, developed more slowly, and decreased less after weaning. Reticular cell reactions in sections incubated at more acid pH values were diminished, and at pH 2.2 only epithelial staining was noted. Glucuronidase activity was similar to that of galactosidase. Reactions for fucosidase and glucosidase were prominent in reticular cells but positive reactions were confined to the jejunum. The reactions in sections from germ-free animals were, in general, similar to those in specimens from 1-week-old animals.

The histochemical reactions confirm previous data based on homogenate assays. The different pattern of development of glycosidase activity in mononuclear cells, however, suggests the presence of isoenzymes with potentially different functions.

## I. INTRODUCTION

Hydrolysis of dietary disaccharides is necessary for adequate nutrition from oral feeding after birth and after weaning. For this reason the changes in neonatal intestinal enzyme activity are of particular interest. Beta-galactosidase (lactase) is especially important in the neonatal period because lactose is the only significant carbohydrate in breast milk.

Detailed studies of β-galactosidase and β-glucuronidase during gestation and the neonatal period have been reported in several laboratory animals.[1-8] These studies have employed biochemical assays of tissue homogenates. Although some differences have been found among species and in different portions of the intestine, the general pattern of development is that of a rapid rise in enzyme activity after birth and a decrease to adult levels in the late neonatal period. More limited data from assays of human intestine suggest that these enzyme levels are minimal during fetal life and rise rapidly only in late gestation and after birth.[9-12] Indirect increases of β-galactosidase activity from oral lactose tolerance tests have shown that lactose absorption increases markedly in the first week of life and is decreased in the premature compared with the full-term newborn infant.[13,14]

Histochemical techniques using halogen-substituted indolyl substrates have been recently developed for several glycosidases: β-galactosidase (3.2.1.23)*, β-glucuronidase (3.2.1.31), β-glucosidase (3.2.1.31), and β-fucosidase (3.2.1.28). The present study was designed to confirm the changes in enzyme activity during the neonatal period and to describe its tissue localization.

## II. MATERIALS AND METHODS

Specimens were obtained from four to six freshly sacrificed Fischer 344 rats at each of the following ages: newly born, 4, 7, 10, 14, and 28 days. Additional tissues from 21-day fetuses, normal adult, and adult germ-free animals were used. Portions of proximal jejunum, terminal ileum, and colon were immediately frozen in a dry ice and acetone bath and stored at -70 C. Frozen sections were cut in a Harris-International cryostat at -15 C.

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* Enzyme Nomenclature, International Union of Biochemistry, Elsevier Publishing Co., Amsterdam, 1965.

The sections were incubated for 4.5 hours at 37 C (for galactosidase pH 2.1, 3.5, and 5.4; glucuronidase pH 4.8) and at 23 C (for glucosidase pH 5.4 and fucosidase pH 6.2). After incubation, random slides were lightly counterstained with hematoxylin and eosin, and all sections were dehydrated in alcohol and cleared in xylol. The intensity of the reaction was graded from 0 to ++++. Detailed descriptions of the substrates* their specificity, and optimal reaction conditions have been published elsewhere: 5-bromo-4-chloroindole-3-yl-β-D-galactopyranoside,[15] 5-bromo-4-chloroindole-3-yl-β-D-glucopyranoside,[16] 5-bromo-4-chloroindole-3-yl-β-D-glucopyruroniside,[17] and 5-bromo-4-chloroindole-3-yl-β-D-fucopyranoside.**

Tissues from littermates were fixed in 10% neutral buffered formalin. After fixation, the tissues were processed, cut, and stained with hematoxylin and eosin in the usual manner.

## III. OBSERVATIONS

The jejunum, ileum, and colon had a similar morphology at birth and were not easily distinguished in routinely stained sections. By 28 days of age, the histologic appearance resembled that in the adult animal, and the anatomic segments were identified with less difficulty. The development of villi, their cellularity, and other characteristic changes in the jejunum during the neonatal period are illustrated in Figure 1. In the adult germ-free animal, the villi were blunted and there was a paucity of lymphoid tissue. The cell population in the lamina propria increased during the first month of life, and by 28 days numerous fibroblasts (perhaps macrophages), lymphocytes, eosinophilic leukocytes, and endothelial nuclei were seen. No striking changes in the epithelial cells were apparent.

The histochemical reactions were visualized as discrete blue granules and rods; this fine granularity was apparent at higher magnification even in intensely stained cells. The reactions were present in both the epithelial cytoplasm and the mononuclear cells at the lamina propria. Less intense staining in mononuclear cells near the serosa was noted in several sections with all substrates. No diffusion of epithelial cell staining was noted, and only with occasional ++++ mononuclear cells were the exact boundaries of the reaction product poorly defined.

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* The four indolyl substrates were prepared by and are available from Dr. H. Plaut et al., Cyclo Chemical Corporation, 1922 E. 64th Street, Los Angeles, California.

** Unpublished data.

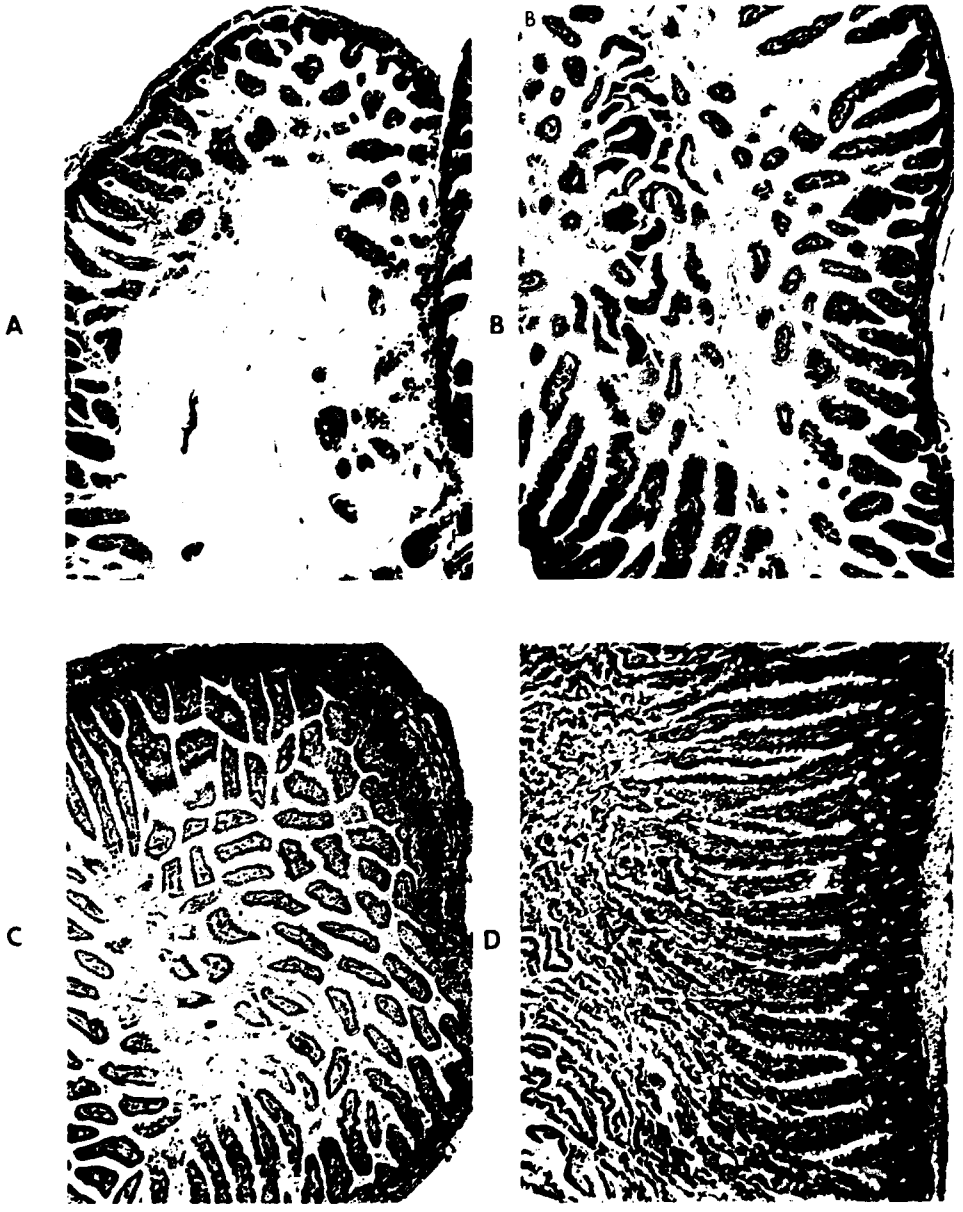


Figure 1. Changes in the Rat Jejunum During Neonatal Period. Intestinal mononuclear cells are present at birth but became increasingly abundant with villous development. A. Newborn, B. 4 days, C. 10 days, D. 28 days. H&E.

Reactions for β-D-galactosidase (pH 5.4) in the epithelial cytoplasm were present in the fetus, but were increased in the early neonatal period. At 28 days, the staining intensity was similar to that in the adult. Representative sections are illustrated in Figure 2. An increase in reactivity was also demonstrated in sections of ileum; the maximal staining occurred at 10 and 14 days. After incubation at more acid pH values (pH 3.5 and 2.1), the neonatal changes in galactosidase activity were identical to that at pH 5.4, although the reaction was less intense. The reticular cells in the lamina propria of the jejunum and ileum stained more intensely than the epithelium. Increased reactivity was again apparent during the first 2 weeks of life, and the number of reactive cells increased greatly during this interval. No mononuclear staining for galactosidase was seen at pH 2.1. Galactosidase reactions were present in the epithelium and mononuclear cells of the colon, but changes with age were less marked.

The histochemical reaction for β-D-glucuronidase was also noted in both cell types. The epithelium of the small intestine did not stain in sections from the fetus, and at birth only a slight (±) reaction was seen in the jejunum. The increase during the first 2 weeks was, however, as marked as that seen for β-D-galactosidase. The reactions in 28-day and adult animals were indistinguishable. The comparison between 10 and 28 days is seen in Figure 3. The pattern of mononuclear cell staining was similar to that of the epithelium. The reactions for β-D-galactosidase and β-D-glucuronidase are listed in Table 1.

Reactions for β-D-glucosidase and β-D-fucosidase differed from the above reactions in that staining was found only in sections of the jejunum. Epithelial glucosidase activity was ++ in the newborn, + at 7 days, and absent in the adult. In contrast, mononuclear cell staining was ++++ during the first 2 weeks and +++ in 28-day and adult animals. Under the conditions employed, the substrate for fucosidase gave the strongest reactions, and ++++ staining was present in epithelial and mononuclear cells during the neonatal period. The adult jejunum showed ++ epithelial and ++++ mononuclear cell staining. Representative sections of these reactions are shown in Figures 4 and 5. Focal staining for glucosidase and galactosidase was present in meconium in several sections. This was interpreted as reactivity of sloughed epithelial cells.

The results in sections of jejunum from germ-free animals resembled those in the neonatal jejunum for all substrates. In contrast, the reactions in the ileum and colon were similar in germ-free and normal adult animals.

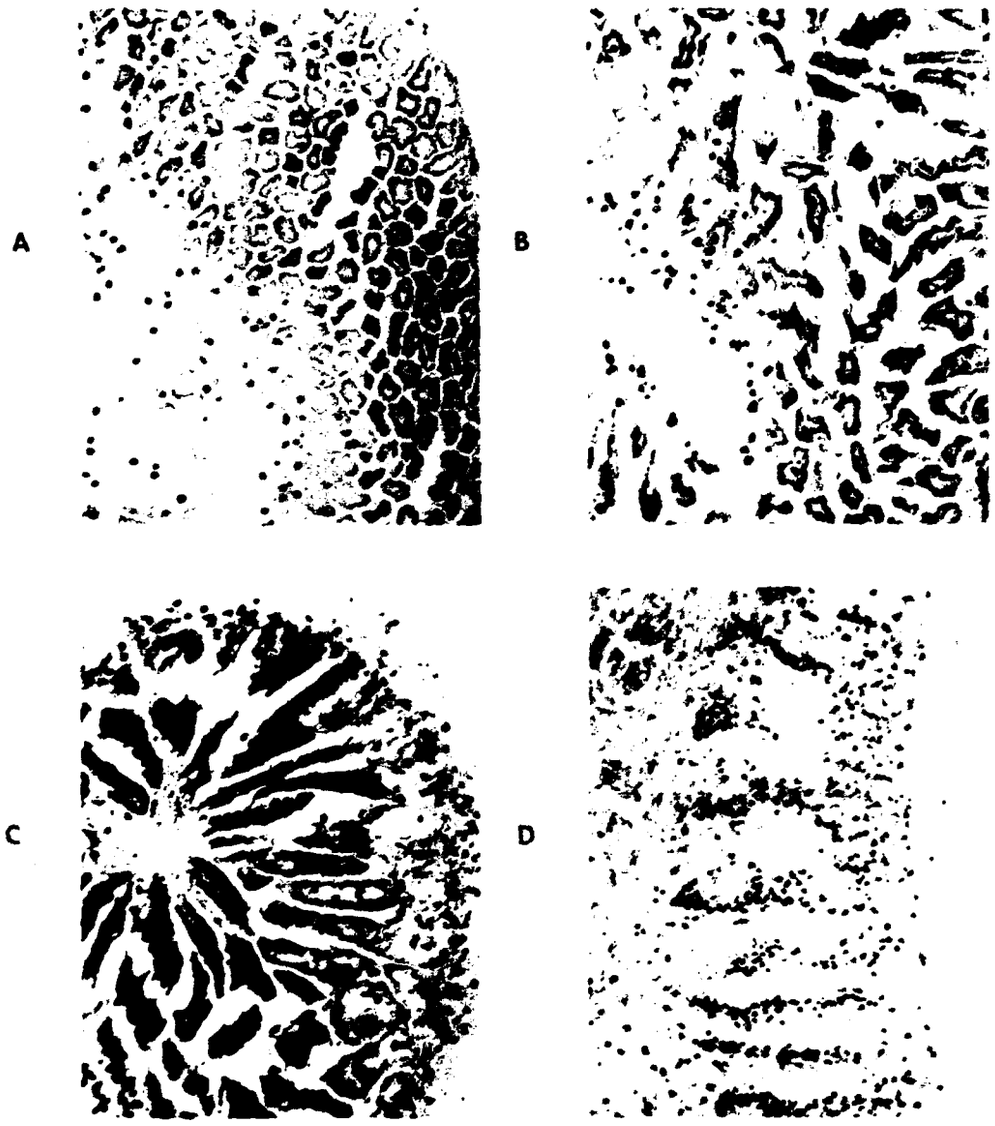


Figure 2. Cryostat Sections of Neonatal Jejunum Incubated with 5-bromo-4-chloroindol-3-yl-β-D-galactopyranoside. Sections show the rapid increase in reactivity of both the epithelium and intestinal reticular cells after birth. The staining intensity at 1 month (D) is similar to that in adult jejunum. A. Newborn, B. 4 days, C. 10 days, D. 28 days. Uncounterstained.

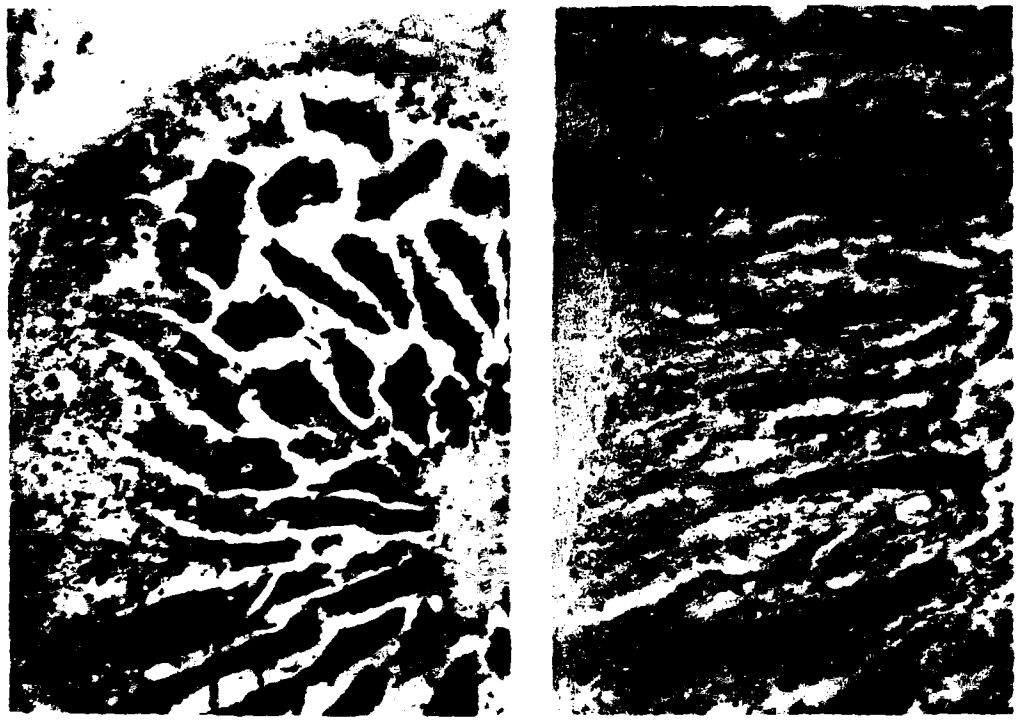

Figure 3. Reactivity for β-D-glucuronidase of Epithelium and Intestinal Mononuclear Cells in the Jejunum. A. 10 days, B. 28 days. Uncounterstained. 95X.

TABLE 1. COMPARATIVE REACTIONS FOR β-D-GALACTOSIDASE AND β-D-GLUCURONIDASE IN THE NEONATAL RAT INTESTINE

| Age, days | Gal, pH 5.4 M[a] | Gal, pH 5.4 R[b] | Gal, pH 3.5 M | Gal, pH 3.5 R | Gal, pH 2.1 M | Gal, pH 2.1 R | GLCR M | GLCR R |
|---|---|---|---|---|---|---|---|---|
| | | | | Jejunum | | | | |
| Fetus | + | ++ | 0 | ++ | 0 | 0 | 0 | 0 |
| Newborn | ++ | +++ | ± | ++ | + | 0 | ± | ± |
| 4 | +++ | ++++ | + | +++ | ++ | 0 | +++ | ++ |
| 7 | ++ | +++ | + | +++ | + | 0 | ++++ | +++ |
| 10 | ++ | +++ | + | +++ | + | 0 | ++++ | +++ |
| 14 | ++ | +++ | ± | +++ | + | 0 | ++++ | +++ |
| 28 | ± | +++ | 0 | ++ | ± | 0 | ++ | +++ |
| Adult | ± | ++ | 0 | ++ | ± | 0 | ++ | +++ |
| Germ-free | ++ | +++ | + | +++ | + | 0 | +++ | +++ |
| | | | | Ileum | | | | |
| Fetus | ± | +++ | ± | ++ | ± | 0 | 0 | 0 |
| Newborn | + | +++ | ± | +++ | + | 0 | 0 | 0 |
| 4 | + | +++ | + | +++ | + | 0 | + | + |
| 7 | ++ | ++++ | + | ++++ | ++ | 0 | +++ | +++ |
| 10 | +++ | ++++ | ++ | ++++ | ++ | 0 | +++ | +++ |
| 14 | +++ | ++++ | ++ | ++++ | ++ | 0 | +++ | +++ |
| 28 | + | ++ | + | +++ | + | 0 | ++ | ++ |
| Adult | + | ++ | ± | +++ | + | 0 | ++ | ++ |
| Germ-free | + | +++ | ± | +++ | + | 0 | ++ | +++ |
| | | | | Colon | | | | |
| Newborn | + | ++ | 0 | ++ | 0 | 0 | + | + |
| 7 | ++ | ++++ | + | +++ | 0 | 0 | +++ | +++ |
| Adult | + | +++ | ± | +++ | 0 | 0 | ++ | +++ |
| Germ-free | + | ++ | ± | +++ | 0 | 0 | +++ | +++ |

a. M - epithelial cells.
b. R - mononuclear cells in lamina propria.

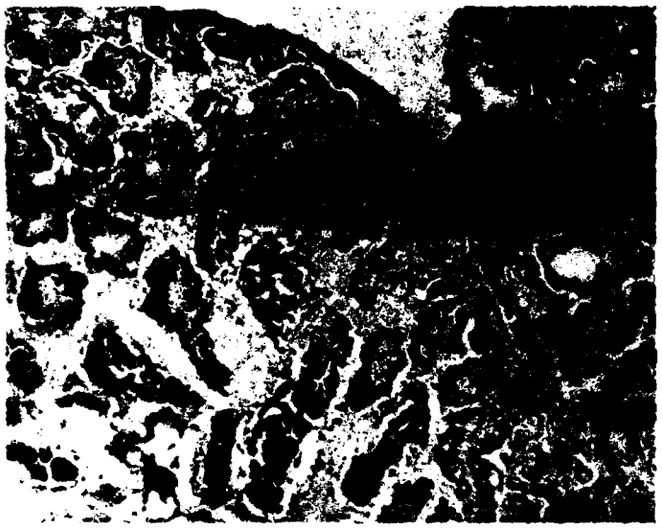

Figure 4. Section of Jejunum Incubated with Indolyl Substrates for β-D-glucosidase. Staining in the newly born animal. Uncounterstained. 130X.

Figure 5. Most Intense Staining Reactions were Those for β-D-fucosidase in the Newborn. Reactivity limited to the jejunum. Uncounterstained. 95X.

## IV. COMMENT

The results of this histochemical study confirm the rapid changes in intestinal β-glycosidase activity in the neonatal period described in the previously cited reports based on biochemical techniques. Histochemistry, furthermore, offers the unique advantage of tissue localization. Staining reactions for galactosidase were less intense at relatively acid pH values and reticular cells failed to stain at pH 2.1. This is the first demonstration of such a change in distribution with pH at the tissue level and supports the concept of multiple, closely related galactosidases.[2,5,8,18]

The rise and fall in galactosidase activity coincides with the beginning of infant feeding and weaning. This temporal relationship has suggested an induced phenomenon to several investigators. Although the data on prolonged lactose ingestion and steroid changes are to some extent conflicting, it is generally conceded that true induction is not operative in the neonatal intestine.[1,2,7,19]

β-Galactosidase is necessary for lactose absorption, but the function of β-glucuronidase in intestinal epithelium is less obvious. These enzymes are present in lysozomes[20] and have been found in both alveolar macrophages and peritoneal mononuclear cell exudates.* The intense activity in reticular cells in the lamina propria is evidence for the resemblance of these intestinal mononuclear cells to circulating and pulmonary phagocytic cells. The strong reactions for β-glucosidase and β-fucosidase in these cells, and their restriction to the jejunum, are of unknown significance.

## V. SUMMARY

Indolyl substrates for β-D-galactosidase, β-D-glucuronidase, β-D-fucosidase, and β-D-glucosidase have been used to detect specific enzymatic activity in frozen sections of proximal jejunum, terminal ileum, and colon. The animals ranged in age from newly born to 28 days. Additional specimens were obtained from fetal, normal adult, and adult germ-free rats.

* Unpublished data. D.J. Yarborough, A.M. Dannenberg, B. Pearson, O.T. Meyer.

Reactions for all enzymes were demonstrated both in the epithelial cytoplasm and in reticular cells of the lamina propria. In the jejunum, minimal reactivity for galactosidase (pH 5.4) in epithelial cytoplasm was present at birth, but increased to high levels by the 4th day. After the 14 day it decreased again to adult levels. Prominent staining of the mononuclear cells of the lamina propria was noted from late gestation through the neonatal period; it decreased somewhat thereafter. Concurrently, the number of reactive cells greatly increased. The reactions of epithelial cells and reticular cells of the lamina propria in sections of ileum were stronger, but the changes with age were similar to those in the jejunum. In contrast, galactosidase activity in the colon was less intense, developed more slowly, and decreased less after weaning (14th day). The reticular cell reactions in sections incubated at more acid pH values were diminished, and at pH 2.2 only epithelial staining was noted. Glucuronidase activity was absent at birth, but after the 4th day followed the same pattern as galactosidase. Reactions for fucosidase and glucosidase were prominent in reticular cells and confined to the jejunum. During the neonatal period no significant changes in staining intensity were seen, but the number of reactive cells increased. The reactions in sections from germ-free rats were, in general, similar to those in specimens from 1-week-old animals.

The histochemical reactions in the epithelial cells are in agreement with previous observations from homogenates of intestinal mucosa. The different pattern of development of glycosidase activity in reticular cells however, suggests a different, and perhaps immune, functional significance for these enzymes in reticular cells in the intestine.

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